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“Analyzing the interactions of Elovanooids with GPR120 in ABC Retinal Pigment Epithelial cells by performing Immunocytochemistry and Incucyte live cell imaging.”

Dry age-related macular degeneration (AMD) is caused by the chronic degeneration of the macula due to drusen deposits in the retina and is the most common cause of vision loss in people over the age of 50. The cause of dry AMD is still unknown but may be caused by the depletion of protective lipids in the retina, which increases oxidative stress in the retina. Treatments for dry AMD are limited but recently discovered neuroprotective lipid mediators, Elovanooids (ELVs), may be useful in combating AMD. ELVs are very long-chain polyunsaturated fatty acids (VLC-PUFAS) that have been hydroxylated to have two alcohol groups. ELVs have been found to protect Retinal Pigment Epithelial (RPE) cells in the retina but the mechanism of this protective effect is unknown.

We are investigating the mechanism of Elovanooid's protective effect on RPE cells using immunocytochemistry (ICC) and Incucyte live cell imaging. Our experiment investigates whether ELVs interact with G-Protein Coupled Receptor 120 (GPR120) using different visualization methods in RPE cells. 8-well chambers were prepared at a density of 15K cells/well. 2 wells were supplemented with ELV-32, 2 were given GPR120 antagonist (AH 7614), and 2 were given GPR120 agonist (GSK137647). ICC was performed to visualize GPR120 surface expression after varying levels of stimulation with each ligand.

Incucyte live cell imaging was used to test the impact of ELVs on RPE cells and the lipid's ability to protect or prevent damage under uncompensated oxidative stress (UOS). 96 well plates of cells were given varying concentrations of hydrogen peroxide while also given ELVs, known antioxidants, Omega-3 fatty acids, and controls to investigate the effect of ELVs on cells with varying levels of uncompensated oxidative stress. The same treatment was used on GPR120 silenced, non-transfected, and negative control RPE cell lines to investigate and compare results to elucidate the role of ELVs on RPE cells in inactivated GPR120. The purpose of these experiments is to determine if ELVs influence the activation or inhibition of GPR120 to aid their protective abilities in RPE cells and determine if GPR120 is pro- or anti-apoptotic. We are investigating GPR120's role in RPE cell protection and if the receptor binds ELVs to better protect against UOS.